Low-magnesium induced long-term potentiation in the rat dentate gyrus in vitro

Melchers, B.P.C.; Pennartz, C.M.A.

Published in:
Neuroscience research communications

LOW MAGNESIUM-INDUCED LONG-TERM POTENTIATION IN THE RAT DENTATE GYRUS IN VITRO

B.P.C. Melchers and C.M.A. Pennartz

University of Amsterdam, Department of General Zoology, Biological Centre, Kruislaan 320, 1098 SM Amsterdam, NL

(Accepted August 11, 1987)

SUMMARY

The effect of a 20 min perfusion with a Ringer solution without Mg^{2+} on the field potentials in the dentate gyrus, was an increase of both the rising slope of the EPSP and of the population spike (PS). After 15-18 min multiple PSs were evoked. The multiple PSs, but not the increase in the EPSP slope and in the first PS, were prevented by the N-methyl-D-aspartate (NMDA) antagonist 2-aminophosphonovalerate (2-APV). After returning to the normal Ringer containing 2 mM Mg^{2+} the field potentials decreased only slightly and remained larger than control values, in case no 2-APV was present during the perfusion without Mg^{2+}. Thus long-term potentiation was induced. However, this was not the case when 2-APV was present during the low Mg^{2+} period. This suggests that the long-term effects are dependent on NMDA-receptor activation.

KEY WORDS: N-methyl-D-aspartate, 2-aminophosphonovalerate, long-term potentiation, dentate gyrus, hippocampal slice, low Mg^{2+}

INTRODUCTION

The use of specific antagonists had led to the conclusion that the NMDA receptor does not contribute to normal synaptic transmission in the hippocampus, evoked by low frequency stimulation (< 0.25 Hz). The receptor may however be of importance in the response to high frequency stimulation (> 10 Hz) which evokes a form of synaptic plasticity that has been called long-term potentiation (LTP). The induction of LTP is prevented by NMDA receptor antagonists (1,2). This suggests a specific role of the NMDA-receptors in the induction of LTP. At normal extracellular Mg^{2+}-concentrations ($[Mg^{2+}]_o$ 1-2 mM) the NMDA receptor-associated ion channels are blocked by Mg^{2+}. This block can be relieved if a sufficient level of depolarization of the cell membranes is reached (3), for instance by a high frequency train of pulses (tetanus), or when the $[Mg^{2+}]_o$ is kept low. Under conditions of low $[Mg^{2+}]_o$ the channels can be activated at normal membrane potentials (4). Reduction of the $[Mg^{2+}]_o$ facilitates, via NMDA receptor-mediated processes,

0893-6609/87/020113-06 $05.00

repetitive burst discharges (4,5) and can lead to spontaneous epileptiform discharges in CA_1 and CA_3 of hippocampal slices (4,6,7).

If NMDA receptor activation is a critical step in LTP induction, this process may be facilitated under conditions of low $[Mg^{2+}]_o$. Under such conditions even low frequency stimulation (0.1-0.25 Hz), which under normal circumstances is not enough to produce LTP, may be sufficient. Indeed, Coan and Collingridge (5) mention that the population spike in area CA_1 of hippocampal slices does not fully recover to control values in many cases after exposure to Mg^{2+}-free media. However, no quantitative description of this phenomenon has yet been given. It is a particular interest to test whether low Mg^{2+} has the same effect on the dentate gyrus, a structure which is also rich in NMDA-receptors (8), since it has been shown that exposure to low concentrations of Mg^{2+}, which produces spontaneous epileptiform activity in area CA_1 and CA_3, fails to do so in the dentate gyrus (7,9). Furthermore, the plastic properties of the dentate gyrus and CA_1 may differ, as is suggested by the different characteristics of Ca^{2+}-induced LTP in both structures (10).

In the present study, we show that "low Mg^{2+}-induced LTP" can occur in the dentate gyrus, a process in which the NMDA receptors are involved. In addition, we give here for the first time a quantitative description of the phenomenon.

MATERIALS AND METHODS

Hippocampal slices (500 µm) were prepared from adult male Wistar rats as described in more detail elsewhere (10). The slices were transferred to the recording chamber, which was constantly perfused (2-3 ml/min) with Ringer solution containing 124 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgSO_4$, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$ and 10 mM glucose, saturated with 95% O_2 and 5% CO_2, at a temperature 33-34°C. The slices were allowed at least one h of rest before the start of an experiment. A number of slices was stored for later use, at room temperature, in a storage chamber, containing Ringer, saturated with 95% O_2 and 5% CO_2.

Stimulation electrodes, 60 µm, insulated stainless steel wires, were positioned in the perforant path. Stimulus pulses, consisting of biphasic, bipolar constant current pulses of 0.2 msec, were delivered to the perforant path with an interval varying between 8-30 sec. The intensity of the pulses was halfway between threshold and the intensity that elicited a saturated response (range: 50-150 µA). For recording two glass micropipettes filled with 3 M NaCl (resistance 3-10 M ohm) were used, which were placed in the stratum granulosum and the stratum moleculare. From the stratum granulosum recordings the population spike was quantified, the stratum moleculare recordings were used to determine the EPSP rising slope. The evoked field potentials were sampled at 4 kHz and stored on diskette for further analysis by means of a Motorola Exorset microcomputer.

After control for a stable baseline, the perfusate was changed to a Ringer without $MgSO_4$ (low Mg^{2+} Ringer), for a period of 20-25 min. During the whole period, the perforant path fibers were stimulated every 8-30 sec with single pulses to evoke field potentials. When desired 2-APV (Cambridge Research Biochemicals) was added in a concentration of 30 µM to the perfusate.

RESULTS

During the perfusion with low Mg^{2+} Ringer both the rising slope of the EPSP

and the population spike (PS) increased in all 10 slices, respectively to 198 ± 12% (SEM) and 252 ± 46% of the response amplitude measured during the control period ($P < 0.01$, Wilcoxon matched pairs signed rank test; Fig. 1).

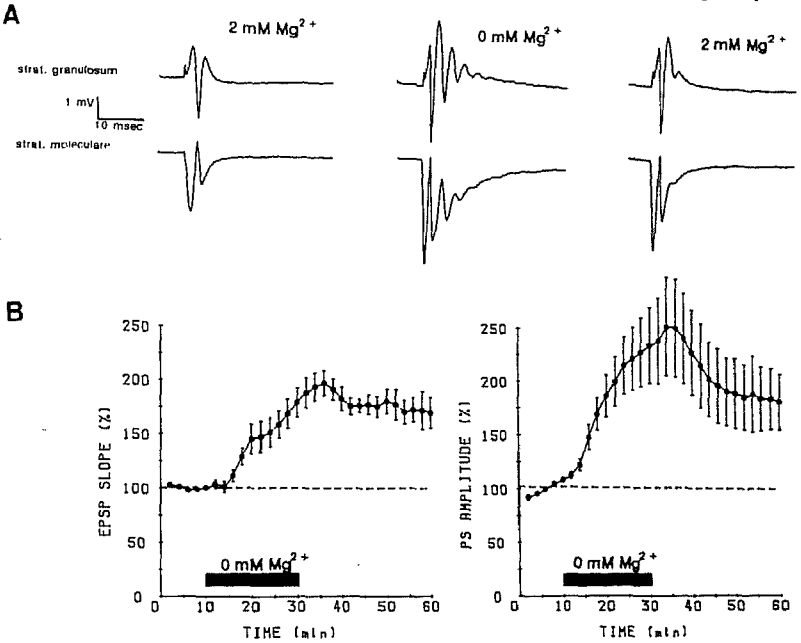

Fig. 1A. Field potentials recorded in stratum granulosum (upper row) and stratum moleculare before, during and 30 min after a period of perfusion with low Mg^{2+} Ringer. B. Average amplitude (mean ± SEM, n = 10 slices), relative to the response during the control period (100%), of the population spike (PS) (right) and the rising slope of the EPSP.

This resulted, 15-18 min after the start of the perfusion with low Mg^{2+}, in an increase of the EPSP and in the development of multiple PSs, indicating an activation of NMDA receptor-associated ion channels (4). After returning to 2 mM Mg^{2+} Ringer the responses changed in the following way. The multiple spikes disappeared in all slices, but in 8 out of 10 slices a second PS remained visible (Fig. 1). The rising slope of the EPSP and the PS amplitude decreased slightly to respectively 169 ± 14 (EPSP slope) and 181 ± 26 (PS) of controls but remained significantly larger than control values at 30 min after returning to normal Ringer ($P < 0.01$, Wilcoxon). In 4 slices the responses were recorded for more than 50 min after returning to normal Ringer, and remained stable during the whole period.

Afferent stimulation appears to be necessary for this long-term effect: two slices which received no afferent stimulation during a first perfusion with low Mg^{2+} Ringer, showed also no LTP (compare Fig. 2A and B). After a second perfusion, during which the slices did receive afferent stimulation (once every 8 sec; Fig. 2C), a long-lasting increase of the evoked field potentials was observed (Fig. 2D).

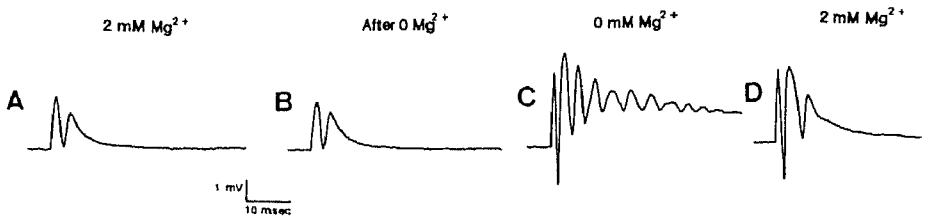

Fig. 2. Field potentials recorded in the stratum granulosum before (A) and 35 min after a period of perfusion (25 min) with low Mg^{2+} Ringer (B) where no afferent stimulation was given and no LTP was observed. The protocol was repeated but now test stimuli were applied during the perfusion with low Mg^{2+} (C). LTP was clearly induced (compare D with A).

In 5 additional slices it was tested which part of the changes could be attributed to NMDA receptor activation. After a stable baseline was established, the Ringer was changed to low Mg^{2+} Ringer, but now containing 30 µM 2-APV. During the perfusion both the rising slope of the EPSP and the PS amplitude increased in all 5 slices respectively to 178 ± 19% and to 223 ± 75% of control (Fig. 3). However, in none of the slices multiple spikes were elicited. After returning to 2 mM Mg^{2+} Ringer the responses decrease to a level just above, but not significantly different from control, averaging for the EPSP rising slope 114 ± 6% and for the PS amplitude 113 ± 4%. During a second period of perfusion with low Mg^{2+} Ringer, now without 2-APV, an increase of both EPSP slope and PS amplitude was found, on average to 330 ± 51% of control level for the EPSP slope and to 285 ± 63% for the PS. After 15-18 min perfusion with low Mg^{2+}, multiple spikes developed in all slices. After returning to 2 mM Mg^{2+}, the responses decreased, stabilizing at relatively high average level of 225 ± 25% for the EPSP rising slope and 239 ± 36% for the PS amplitude, at 30 min after returning to 2 mM Mg^{2+}. Multiple spikes disappeared, but in 4 slices a second PS remained present (Fig. 3A). In 4 slices it was tested whether this second PS was sensitive to 2-APV. In all 4 the second PS was reversible abolished by 30 µM 2-APV.

To test whether the LTP induced by the period of perfusion with low Mg^{2+} had reached a maximal value of potentiation, a tetanus (50 Hz, 2 sec, with an intensity which was the same as used for the test stimulus), was applied to the perforant path in 11 slices 35 min after returning to 2 mM Mg^{2+}. The tetanus induced only slight changes in the field potentials. On average (n = 11) the LTP effect elicited only by the low $[Mg^{2+}]_o$ accounted for as much as 99 ± 2.9% (for the EPSP) and 103 ± 4.0 (for the PS) of the total LTP after both the low $[Mg^{2+}]_o$ period and the tetanus. This indicates that LTP was already saturated after the period with low $[Mg^{2+}]_o$. Thus low $[Mg^{2+}]_o$ induces LTP presumably through the same mechanisms as a tetanus.

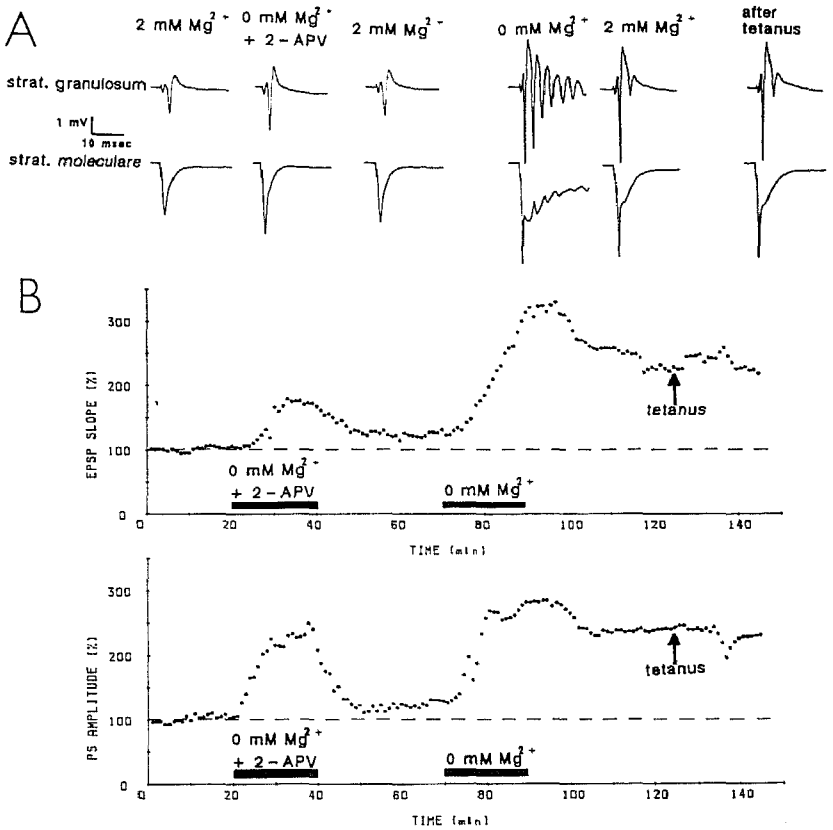

Fig. 3A. Field potentials recorded in the stratum granulosum (upper row) and stratum moleculare; from left to right: before, during perfusion with low Mg^{2+} Ringer containing 30 µM 2-APV, after returning to 2 mM $[Mg^{2+}]_o$, during a second period of perfusion with low Mg^{2+} Ringer now without 2-APV, 30 min after returning to 2 mM $[Mg^{2+}]_o$, and 20 min after tetanization of the perforant path. B. Average (5 slices) of the relative amplitude normalized to the response during the control period (100%) of the rising slope of the EPSP (upper graph) and of the population spike (PS). The periods of perfusion with low Mg^{2+} Ringer are indicated by the black bars; at the point in time indicated by an arrow a tetanus was given to the perforant path. (SEMs are left out for reasons of clearity).

DISCUSSION

It can be concluded that, as was also noted for the CA_1 area (5), a short period with low $[Mg^{2+}]_o$ can lead to large, saturated LTP of evoked field potentials in the dentate gyrus of the hippocampal slice, provided that afferent stimulation is given. In many slices even an NMDA receptor-mediated component in the evoked response, in the form of a second PS, is induced. This may be explained by a reduced inhibition due to a massive NMDA receptor activation, which has been described recently after repeated tetanization of afferents in the hippocampal slice (11). A participation of NMDA receptors in synaptic transmission has been shown following kindling epileptogenesis in the dentate gyrus (12), which may indicate that partly the same processes are involved in LTP induction and in

kindling epileptogenesis. The long-term effects of the perfusion with low Mg^{2+} Ringer appear to be dependent on the activation of NMDA receptors, since i) afferent stimulation is necessary for the effect and ii) they are blocked largely by 2-APV. As was also described by Hamon et al. (13) in the CA_1 area, only part of the effects of low $[Mg^{2+}]_o$ appear to be mediated via the NMDA receptors. Although 2-APV prevents the development of multiple spikes, it fails to block the increase of the EPSP and the PS during the perfusion with low Mg^{2+} Ringer. The reversible increase of both EPSP and PS during low $[Mg^{2+}]_o$ presumably can be explained by a decreased surface charge screening of the neuronal membranes (14) by divalent cations, leading to both a decrease in spike threshold and to an increase in transmitter release.

We wish to thank Fernando Lopes da Silva, Wim Ghijsen and Wytse Wadman for helpful comments during the preparation of the manuscript. This work was subsidized in part by the Dutch Organization for the Advancement of Pure Research (ZWO-Medigon grant 900-548-072).

REFERENCES

1. Collingridge, G.L., Kehl, S.L. and McLennan, H. (1983) J. Physiol. (Lond.) 334, 19.
2. Harris, E.W., Ganong, A.H. and Cotman, C.W. (1984) Brain Res. 323, 132.
3. Nowak, L., Bregestovski, P., Ascher, P., Herbet, A. and Prochiantz, A. (1984) Nature (Lond.) 307, 462.
4. Herron, C.E., Lester, R.A.J., Coan, E.L. and Collingridge, G.L. (1985) Neurosci. Lett. 60, 19.
5. Coan, E.J. and Collingridge, G.L. (1985) Neurosci. Lett. 53, 21.
6. Mody, I., Lambert, J.D.C. and Heinemann, U. (1987) J. Neurophysiol. 57, 869.
7. Walther, H., Lambert, J.D.C., Jones, R.S.G., Heinemann, U. and Hamon, B. (1986) Neurosci. Lett. 69, 156.
8. Monaghan, D.T. and Cotman, C.W. (1985) J. Neurosci. 5, 2909.
9. Stanton, P.K. Jones, R.S.G., Mody, I. and Heinemann, U. (1987) Epilepsy Res. 1, 53.
10. Melchers, B.P.C., Pennartz, C.M.A. and Lopes da Silva, F.H. (1987) Neurosci. Lett. 77, 37.
11. Stelzer, A., Slater, N.T. and ten Bruggencate, G. (1987) Nature (Lond.) 326, 698.
12. Mody, I. and Heinemann, U. (1987) Nature (Lond.) 326, 701.
13. Hamon, B., Stanton, P.K. and Heinemann, U. (1987) Neurosci. Lett. 75, 240.
14. Frankenhaeuser, B. and Hodgkin, A.L. (1957) J. Physiol. (Lond.) 137, 217.